PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : B01L 3/02, G01N 1/14

A1

(11) International Publication Number: WO 98/26872

(43) International Publication Date: 25 June 1998 (25.06.98)

(21) International Application Number: PCT/US97/22654

(22) International Filing Date: 11 December 1997 (11.12.97)

(30) Priority Data:
08/768,656 18 December 1996 (18.12.96) US

(71) Applicant: ANSYS, INC. [US/US]; 2 Goodyear, Irvine, CA 92618 (US).

(72) Inventor: BLEVINS, Dennis, D.; 24952 Wilkes Place, Laguna Hills, CA 92653 (US).

(74) Agent: HACKLER, Walter, A.; Suite B, 2372 S.E. Bristol, Newport Beach, CA 92660 (US).

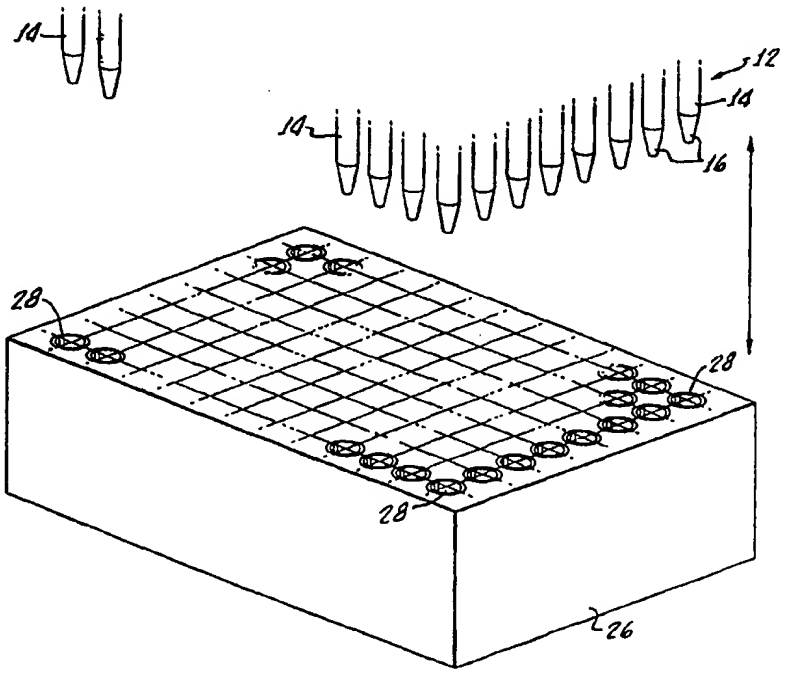
(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: METHOD FOR CONDUCTING ASSAYS AND SEPARATIONS FOR COMPONENTS OF INTEREST IN FLUID SAMPLES

(57) Abstract

A method is provided for conducting assays and separations of components of interest in the fluid samples which includes the steps of providing a plurality of pipettes disposed in an array for the transfer of fluid samples and disposing a solid phase extraction medium in each of the pipettes. A collection tray (26) is provided which includes a plurality of collection compartement (28) disposed in an array (12) for receiving fluid samples. The pipette array (12) is disposed over the collection tray (26) and bidirectional transfer of fluid samples through the pipettes (14) and extraction medium into and out of the collection compartments (28) is performed.



METHOD FOR CONDUCTING ASSAYS AND SEPARATIONS FOR COMPONENTS OF INTEREST IN FLUID SAMPLES

The present invention is generally related to a method for conducting assays for components of interest in fluid samples. More particularly the present invention is directed to a method for the testing and/or analysis of fluid samples usually of a chemical, biochemical or biological nature.

Fluid samples, for example, a solution, colloidal dispersion, or suspension may be separated into individual components through the use of extraction media.

A typical device is set forth in U.S. Patent No. 5,264,184 describing devices for separating liquid samples into components which include a container and a separation layer abutting a bottom wall of the container with an outlet opening formed therein. A vacuum is utilized to pass fluids through the separation layer which are caught drop-by-drop in a collecting container.

A plurality of such sample containers and collection containers may be arranged closely adjacent to one another so that a simultaneous separation of a plurality of liquid samples is made possible. The sample containers may be arranged, for example, side-by-side in a single row and interconnected, or they can be arranged in rows and columns in a two-dimensional matrix and interconnected with the columns being orthogonal to the rows.

This arrangement and many other automated methods and instruments have been developed over the years to perform analysis of fluid samples.

As hereinabove noted, because there is a need for analysis of a great number of small quantities of liquid, automated analytical instruments heretofore developed include a plurality of containers for holding fluid samples to be tested in containers for holding the desired reagents.

As set forth in U.S. Patent 5,417,923, a conventional assay technique is to locate the separation medium in an upstanding tubular chamber having an open top and a bottom outlet and to pipette or otherwise deposit the liquid under test into the chamber to flow through or over the extraction medium prior to emerging from the bottom outlet. U.S. Patent 5,417,923 teaches an assay tray assembly which includes a plastic molded test tray removably mounted in an overlying relationship in engagement with a plastic molded collection tray. The test tray has a spaced array of discrete identical chambers to accommodate a predetermined volume of liquid for analysis with each chamber being formed with a top opening and a bottom opening for flow of liquid or air through the separation media.

The bottom opening is located in a downward projecting tubular spigot for the respective chamber and each chamber carries a separation medium to which the liquid is to be subjected. After passing through the assay tray assembly, the liquid passes into the individual chambers formed in the collection tray.

Many assays require that the medium, or extraction complex, be subjected to one or more isolations, washings and treatments with liquid reagents. Accordingly, to facilitate these procedures, a solid phase extraction medium is used which results in the complexes formed also being in the solid phase. This facilitates the washing and treatment of the solid phase.

As hereinabove noted, U.S. 5,417,923 teaches the use of the separate assay tray assembly along with a corresponding collection tray. The assay trays typically have a plurality of wells, for example, ninety-six (96), arranged in rows and columns into which the particulate solid phase is placed and treated sequentially with the liquid reagents and washes involved in the assay of interest.

A great number of well configurations have been used and suggested as is noted in U.S. Patent 5,417,923.

Fluids are deposited in the assay tray by means of apparatus for handling a plurality of pipettes each aligned for depositing fluids in the assay tray.

Naturally, because of the necessity to isolate each of the assays being performed in a 96-compartment assay tray with corresponding collection trays, exact and perfect alignment of these two components is absolutely required. It is also necessary to design the nesting components so that fluid cannot be transferred from one compartment to another in either the assay tray or the collection tray, nor can fluid be transferred from a compartment in an assay tray to a compartment in the collection tray, which is not intended to be aligned for fluid transfer.

Thus, while the heretofore-described procedures for performing simultaneous multiple assays has been the standard for many years, the multiple components (namely the pipettes, assay trays and collection trays) can result in inaccurate assays due to contamination as hereinabove noted.

Therefore, the object of the present invention is to eliminate the need for the assay tray in conducting multiple simultaneous assays.

SUMMARY OF THE INVENTION

An assay or liquid separation method in accordance with the present invention for components of interest in fluid samples generally includes the steps providing a plurality of pipettes disposed in an array for the transfer of fluid samples along with disposing a solid phase extraction medium in each of the pipettes. With regard to liquid evaporation, the method in accordance with the present invention also provides for preparing samples for subsequent analysis by separating liquid samples into their individual components.

A collection tray is provided having a plurality of collection compartments disposed in an array for receiving fluid samples. The method further includes a step of positioning the pipette array over the collection tray and directly transferring fluid

samples through the pipettes and extraction medium therein into the collection compartment.

In this manner, the use of a test tray, hereinabove described in connection with the prior art, is totally eliminated. Consequently, the method in accordance with the present invention provides for a more efficient and economical assay.

More particularly, and importantly, the method in accordance with the present invention may include the step of withdrawing fluid sample directly from the collection compartments and through the pipette and extraction medium therein. Thus, transferring fluids through the extraction medium may be effected by both passing fluid from the pipettes into the compartments and by passing fluid from the compartments into the pipette. Naturally, this bidirectional fluid sequence through the pipette and extraction medium may be iterated any number of times with various fluids to enable a great number of procedures to be conducted, if desired, during an assay or liquid separation preparation for a subsequent assay.

In that regard, the method of the present invention may also include the step of providing a second collection tray having a plurality of collection compartments disposed in an array, each with a second fluid sample therein, and directly transferring the second fluid samples through the pipettes and extraction medium therein. The method in accordance with the present invention may further comprise a step of providing a second plurality of pipettes disposed in an array, disposing a solid phase extraction medium in each of the pipettes in the second array and directly transferring the second fluid samples through the pipettes in the second array and extraction medium therein.

While the number of pipettes and compartments may not be equal in number, the method in accordance with the present invention also includes the step of providing a collection tray with an array of compartments of equal number to the number of pipettes in the pipette array. In this regard, the compartments and pipettes have identical arrays.

Alternatively, in accordance with the present invention, a method for performing an assay for components of interest in a fluid sample may include providing a plurality of pipettes disposed in an array for transfer of fluid samples and disposing a solid phase extraction medium in each of the pipettes. A collection tray is provided with a plurality of collection departments disposed in an array and a fluid sample disposed in each of the compartments.

The method then includes positioning the pipette over the collection tray and directly transferring the fluid samples from the compartments into the pipettes through the extraction medium. This particular embodiment of the method of the present invention provides for initial movement of fluid samples from the compartments into the pipettes and through the extraction medium, as opposed to the initial transfer of fluid samples from the pipette through the extraction medium and into the compartments.

Any combination of the hereinabove noted procedures also should be considered to be within the scope of the present invention.

BRIEF DESCRIPTION OF THE DRAWINGS

The advantages and features of the present invention would be better understood by the following description when considered in conjunction with the accompanying drawings in which:

Figure 1 shows a perspective view of an array of pipettes and a collection tray having a corresponding array of compartments therein; and

Figure 2 is a cross-section of a portion of pipette suitable for use in the method of the present invention showing a solid phase extraction medium therein.

DETAILED DESCRIPTION

The technology of solid phase extraction is well-explained in the literature and has been the topic of numerous patents. Although specific terms are used to describe the invention, techniques that operate equivalently are included. The solid phase extraction experiment involves the selective sorption of components from a

mixture by, most commonly, passing the mixture through the extraction media. If desired, the sorbed analytes are removed from the extraction media by passing another solvent, having select different properties compared to the sample, through the extraction media.

Turning now to Figure 1, there is shown an array 12 which includes a plurality of pipettes 14 held in the array 12 by a fixture (not shown) which also provides a means for forcing a fluid sample (not shown) out of tips 16 of the pipettes 14 and through a solid phase extraction disc, or medium, 20 disposed within each pipette 14. (See also Figure 2.) Alternatively, fluid sample may be drawn through the tip 16 and the extraction medium 20 into a frusta conical tip 22 of the pipette with sufficient force to prevent dislodging thereof when fluid flow is from outside of the pipette 14 into a body 24 thereof. The extraction medium 20 is preferably press fit.

It should be appreciated that any suitable fixture for holding the pipettes in an array and providing fluid samples thereto may be utilized. Alternatively, the fluid samples may be predisposed into the pipettes before assembly into the array 12, then supported by the fixture (not shown) which may provide air pressure for movement of fluid samples through the pipette in the directions hereinabove described.

Also shown in Figure 1 is a collection tray 26 which includes a plurality of compartments 28 therein. Any number of pipettes 14 and compartments 28 may be provided, for example, ninety-six (96), disposed in an array of 8 x 12. The array 12 includes the pipettes 14 disposed in a matching array with the compartments 28 of the collection tray 26. However, it should be appreciated that unequal numbers of pipettes 14 and compartments 28 may be utilized.

In accordance with the present invention, the pipette array 12 is positioned over the collection tray 26 and fluid samples are passed through the pipettes and the extraction medium into the collection compartment 28.

In that regard, the pipette array 12 is lowered until proximate the collection tray 26 as indicated by the arrow 30 which also shows movement of the pipette array 12 away from the collection tray 12 to facilitate handling of the collection tray which may be subsequently moved to another site for further analysis.

In furtherance of such continued analysis, another pipette array (not shown) which may be identical to the pipette array 12 shown in Figure 1 may be used to further transfer fluids to and from the compartments and through solid extraction media disposed in the pipettes 14.

In addition, a second collection tray (not shown), which may be identical to the collection tray 26 shown in Figure 1, may be utilized and positioned under the pipette array 12 for transfer of fluids from the pipettes 14 into the compartments 28 or vice versa.

In this manner, any combination of steps which may be necessary for a particular assay that includes the use of multiple fluids and multiple extraction media may be formed with the method in accordance with the present invention.

It is contemplated that the method of the present invention would be suitable in the following assays:

EXAMPLES

Example 1

This example illustrates the sorption of a colored dye component from a mixture to allow visualization of the technique. The use of such compounds helps those skilled in the art to understand the retention of specific analytes under defined conditions.

Solid phase extraction (SPE) is a sample preparation technique that employs disposable devices to quickly extract desired analytes from a given sample. Several different types of surface interactions are available for the isolation, depending on the type of media used. SPEC® non-polar extraction media, such as C18, contain

silica particles bonded with hydrophobic groups. Typically, the extraction process begins with a conditioning sequence of methanol followed by an aqueous solution in order to prepare the SPEC® extraction media for use. Once the sample is added, the hydrophobic analyte of interest is retained via hydrophobic interactions with the sorbent. Next, an aqueous wash solution is passed through the SPEC® media to remove sample matrix interference. Finally, the analyte is eluted using an organic solvent, such as methanol.

It should be appreciated that the procedural steps of conditioning, wash and elution solvent addition to the top of the SPEC® simulate using an automated dispensing pipettor. The same results would be obtained by drawing the solvents through the bottom of the SPEC® and reversing the flow to displace the solvent for the conditioning, wash and elution steps not using an automated dispensing pipettor.

To visualize the SPE extraction process, a solution of green food coloring is separated into its components: FD&C Blue #1 and FD&C Yellow #5. The chemical structure of the blue component indicates more hydrophobicity and less functional group ionization, as compared to the chemical structure of the yellow component.

A disc 20 of SPEC® C18 SPE extraction media was inserted and pressed into the disposable pipette tip 22 towards the bottom. The media was held in place by the press fit on the sides of the frusta conical chamber. One tenth (0.1) mL methanol was added to the disc through the opening at the top and pushed through the disc using the pipettor. Then 0.1 mL water was added to the disc in a similar fashion to displace excess methanol. A pipettor (not shown) was attached to the pipette 14 containing the SPEC® disc 20 and the plunger depressed. The tip 22 was then inserted into a compartment 28 containing 0.5 mL of an aqueous solution containing dilute green food coloring (a mixture of the blue and yellow compounds described above). The plunger of the pipettor was slowly released, drawing the colored solution through the disc 20. Whilst the liquid was flowing through the disc 20, the blue component was sorbed on the surface of the disc 20, and the yellow solution was in the pipette tip 22 above the disc 20. The pipette tip 22 was then moved to a clean compartment and the pipettor plunger was depressed, expelling the liquid,

reversing the direction of sample flow through the disc 20. The blue compound remained adsorbed on the extraction disc 20 and the yellow component was in the compartment. To elute the blue component from the disc 20, the pipettor was removed from the pipette 14 containing the extraction media, disc 20, and 0.25 mL methanol was added to the pipette 14 above the disc 20. The pipettor was re-attached, and the tip 22 was placed into a clean compartment. Depressing the plunger on the pipettor caused the methanol to flow through the disc 20, eluting the blue component into the compartment.

Example 2

This example shows an application extracting morphine and codeine from an authentic urine specimen using the SPEC® pipette tip extraction device format.

Urine specimens were spiked with morphine and codeine at three different levels; negative, the SAMHSA cutoff level of 300 ng/mL, and a higher concentration of 750 ng/mL. The experiments were run in triplicate for specimens containing the analytes. The quantitation was based on a relative recovery experiment with the deuterated internal standards added to the urine specimen.

The method used was a modification of the published SPEC® extraction procedure for the extraction of morphine and codeine from urine specimens. The samples consisted of 0.5 mL spiked urine, internal standards, and a bicarbonate buffer so that the final volume was 0.75 mL, The pipette tips were constructed in a similar fashion to the device outlined in Example 1, except using the SPEC® MP1 (mixed phase 1) extraction media instead of the C18 media. The MP1 extraction media contains both hydrophobic and cation exchange moieties.

The procedural steps for processing the specimens follow. The columns were conditioned with 0.1 mL methanol. The excess solvent was pushed through the extraction media using the pipettor. With the plunger depressed, the pipette tip containing the conditioned media was lowered into a test tube containing the urine sample. The plunger was released drawing the urine through the extraction media in the pipette tip. After all the urine flowed through the disc, the plunger was

depressed pushing the urine back through the disc into the original test tube. A wash step for the disc inside the pipette tip consisted of the sequential addition of 0.25 mL water, 0.1 M acetic acid, and methanol, ensuring displacement of each solvent before the addition of the next solvent. The analytes of interest were eluted from the disc by the addition of 0.25 mL mixture of ethyl acetate, methanol, and ammonium hydroxide and collecting the solvent into a clean vial. The elution step was repeated one time. The collected solvent was processed in the appropriate manner, including a derivatization step and the final extracts were analyzed using gas chromatography mass spectrometry (GC/MS).

The quantitative results are given in Table I. The data in Table I demonstrate that the pipette tip SPE device and method employing the device performed according to the present invention. In all cases, the expected results were obtained for the morphine and codeine levels spiked.

TABLE I

ANALYTE	SPIKED LEVEL (ng/mL)	DETERMINED LEVEL (ng/mL)
morphine	negative 300 750	none detected 306 733
codeine	negative 300 750	none detected 312 758

Although there has been hereinabove described a particular assay and separation method for components of interest in fluid samples in accordance with the present invention, for the purpose of illustrating the manner in which the invention may be used to advantage, it should be appreciated that the invention is not limited thereto. Accordingly, any and all modifications, variations, or equivalent arrangements which may occur to those skilled in the art, should be considered to be within the scope of the present invention as defined in the appended claims.

WHAT IS CLAIMED IS:

1. An assay method for components of interest in fluid samples, the method comprising the steps of:

providing a plurality of pipettes disposed in an array for the transfer of fluid samples;

disposing a solid phase extraction medium in each of the pipettes;

providing a collection tray having a plurality of collection compartments disposed in an array for receiving fluid samples;

positioning the pipette array over the collection tray; and

directly transferring the fluid samples through the pipettes and extraction medium into the collection compartments.

2. The method according to claim 1 further comprising the step of withdrawing fluid samples directly from the collection compartments and through the pipette and extraction medium therein.

3. The method according to claim 1 further comprising the steps of providing a second collection tray having a plurality of collection compartments disposed in an array, each with a second fluid therein and directly transferring the second fluid from the second tray collection compartment through the extraction medium and into said pipettes.

4. The method according to claim 3 further comprising the step of providing a second plurality of pipettes disposed in an array disposing a solid phase extraction medium in each of the pipettes in the second array and directly transferring the second fluid samples through the pipettes in the second array and extraction medium therein.

5. The method according to claim 1 wherein the step of providing a plurality of pipettes and the step of providing a collection tray with an array of

compartments each include providing an identical array for the pipettes and compartments.

6. The method according to claim 1 wherein the step of providing a plurality of pipettes and the step of providing a collection tray with an array of a plurality of compartments each include providing an equal number of pipettes and compartments.

7. The method according to claim 7 wherein the step of positioning the pipette array over the collection tray includes positioning each pipette over a corresponding component in the collection tray.

8. An assay method for components of interest in fluid samples, the method comprising the steps of:

providing a plurality of pipettes disposed in an array for the transfer of fluid samples;

disposing a solid phase extraction medium in each of the pipettes;

providing a collection tray having a plurality of collection compartments disposed in an array;

disposing a fluid sample in each of the compartments;

positioning the pipette array over the collection tray; and

directly transferring the fluid samples from the compartments into the pipettes through the extraction medium.

9. The method according to claim 8 further comprising the step of directly transferring the fluid samples through the pipettes and extraction medium into the collection compartment.

10. The method according to claim 1 further comprising the step of providing a second collection tray having a plurality of collection compartments disposed in an array, each with a second fluid therein and directly transferring the second fluids through the pipettes and extraction medium.

11. A method for separating components of interest in fluid samples, the method comprising the steps of:

providing a plurality of pipettes disposed in an array for the transfer of fluid samples;

disposing a solid phase extraction medium in each of the pipettes;

bidirectionally passing a fluid sample and a solvent through the pipettes and solid phase extraction medium to form sorbed analytes in the solid phase extraction medium and to subsequently remove the sorbed analytes from the solid phase extraction mediums.

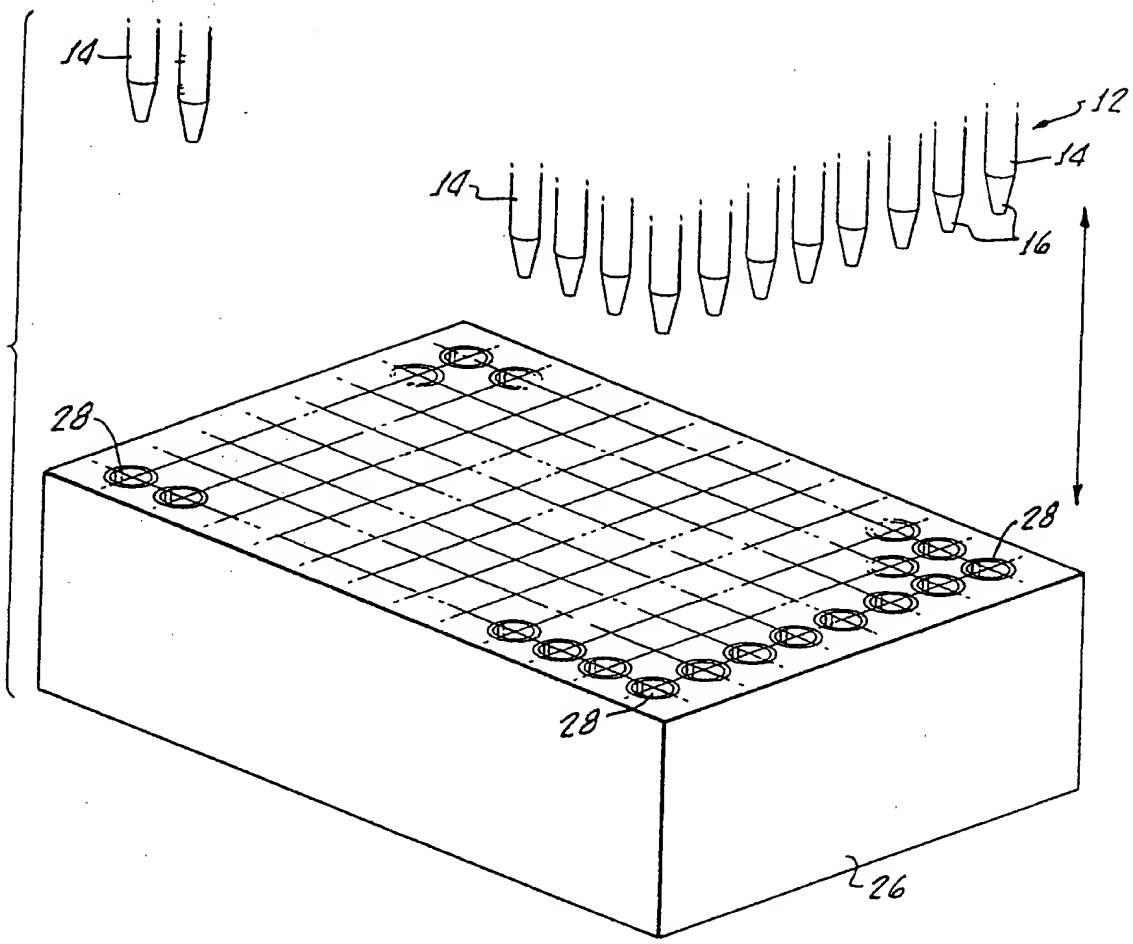


FIG. 1.

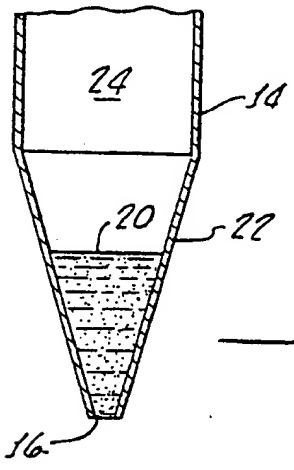


FIG. 2.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/22654

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :B01L 3/02; G01N 1/14
US CL :422/100

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 422/100, 101, 102, 103, 104; 73/864.01, 864.11, 864.17, 864.22

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

NONE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 4,158,035 A (HAASE ET AL) 12 June 1979 (12-06-79), see entire document.	1-11
Y	US 5,156,811 A (WHITE) 20 October 1992 (20-10-92), see entire document.	1-11
Y	US 5,092,184 A (GOODELL ET AL) 03 March 1992 (03-03-92), see entire document.	1-11
A	US 5,496,523 A (GAZIT ET AL) 05 March 1996 (05-03-96), see entire document.	1-11
A	US 5,456,879 A (SUOVANIEMI) 10 October 1995 (10-10-95), see entire document.	1-11
A	US 5,417,923 A (BOJANIC ET AL) 23 May 1995 (23-05-95), see entire document.	1-11

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search
23 MARCH 1998

Date of mailing of the international search report
13 APR 1998

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231
Facsimile No. (703) 305-3230

Authorized officer
HAROLD Y. PYON
Telephone No. (703) 308-0651

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/22654

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5,403,489 A (HAGEN ET AL) 04 April 1995 (04-04-95), see entire document.	1-11
A	US 5,264,184 A (AYSTA ET AL) 23 November 1993 (23-11-93), see entire document.	1-11
A	US 3,982,438 A (BYRD) 29 September 1976 (29-09-76), see entire document.	1-11
A	US 3,807,235 A (LEFKOVITS ET AL) 30 April 1974 (30-04-74), see entire document.	1-11